HARVESTING OF FRESHWATER MICROALGAE BY BACTERIAL BIOFLOCCULANT, NDIKUBWIMANA Theoneste, XIAMEN UNIVERSITY, JUNE 2016

University Code: 10384

Student ID No.: 20620130154400

厦门大学

HARVESTING OF FRESHWATER MICROALGAE BY BACTERIAL BIOFLOCCULANT

利用微生物絮凝剂高效收获微藻

NDIKUBWIMANA Theoneste

Supervisor: Professor Lu Yinghua

Co-Supervisor: Associate Professor Zeng Xianhai

Major: Chemical Engineering

Time of Graduation: June, 2016

A DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMICAL AND BIOCHEMICAL ENGINEERING

COLLEGE OF CHEMISTRY AND CHEMICAL ENGINEERING

XIAMEN UNIVERSITY

CERTIFICATION

I, Professor **Lu Yinghua**, hereby certify that I have read this manuscript and recommend for acceptance by the Xiamen University a dissertation entitled "**HARVESTING OF FRESHWATER MICROALGAE BY BACTERIAL BIOFLOCCULANT**" in fulfillment of degree of **Doctor of Philosophy** at Xiamen University, People's Republic of China.

Signed………………
Supervisor
Date………………
Department of Chemical and Biochemical Engineering
College of Chemistry and Chemical Engineering
Xiamen University
Xiamen, Fujian Province
P.R.China

ORIGINAL STATEMENT

The research described in this Dissertation was conducted under the supervision of Professor Lu Yinghua at the Department of Chemical and Biochemical Engineering, Xiamen University and Associate Professor Zeng Xianhai at College of Energy, Xiamen University. I hereby declare that the work submitted is my own and that appropriate credit has been given where reference has been made to the work of others. I also confirm that it has not been previously or concurrently submitted for any other degree, diploma or any other qualifications at Xiamen University, P.R China or other institutions**.**

NDIKUBWIMANA Theoneste

June, 2016

COPYRIGHT DECLARATION

NDIKUBWIMANA Theoneste

June, 2016

ACKNOWLEDGEMENTS

In all humbleness, I acknowledge the blessings of Almighty God for his Love and Mercy upon me, and for giving me the chance to study in Xiamen University, thus making my dreams a reality. My indebtedness goes to my supervisor Professor Lu Yinghua and Co-supervisor Associate Professor Zeng Xianhai, for their immense input, direction, guidance, advice and motivation. I'm very grateful and proud to be one of their students.

I'm highly grateful to the Government of People's Republic of China, who trough the Chinese Government Scholarship Council (CSC) offered me the full scholarship to study in Xiamen University. I wish to extend my sincere thanks to all the lecturers in the Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, for being the driving force behind my training, their knowledge and skills imparted to me is the key for my success. I'm heartily grateful to the people of China through whose taxes I was offered the scholarship. Specifically, I thank all the past and present members of the research group of Professor Lu Yinghua, for their care, understanding, and ceaseless support.

I would like to express my deepest gratitude to my parents, family and friends, for their encouragement, understanding, motivation and constant support during my stay away far from home. I sincerely express my gratitude to everyone who has contributed in one way or the other in the completion of my studies, I am grateful for your endless help, encouragement and patience.

May the Almighty God, in his countless Mercy, pour his blessing on all those who in diverse ways contributed to my success!

ABSTRACT

In respect to compensate the unremittingly increasing global demand for food, feed, biofuels, and chemicals production, microalgae has been widely regarded as one of the most promising raw materials. However, the small particulate size of microalgae cells and the extremely dilute concentrations of microalgae cultures continue to be the major challenges to effective microalgae harvesting, thus hindering the production and commercialization of microalgae based products. In an attempt to find a cost-effective and environmentally friendly harvesting technique, the bioflocculant poly γ-glutamic acid (γ-PGA) produced by *Bacillus licheniformis* CGMCC 2876 was used to concentrate freshwater microalgae. Zeta potential measurement, and classical DLVO theory (named after Derjaguin, Landau, Verwey and Overbeek) analysis were used to explore the flocculability of microalgae induced by bacterial γ-PGA broth bioflocculant. The process was evaluated for its scalability and *in-situ* treatment feasibility. Furthermore, biochemical composition of microalgal biomass was analyzed to evaluate the applicability of bioflocculation for microalgae based biofuel production. Experimental results showed that the flocculation efficiency was dependent on the initial culture pH. With the optimum operating parameters of bioflocculant dosage of 2.5 mL/L, flash mixing rate of 150 rpm for 1 min, and slow mixing rate of 80 rpm for 2 min, a flocculation efficiency of 99% was achieved at initial culture pH 3 at small scale. The microalgae interaction energy was found to be dependent on the zeta potential. The classical DLVO theory indicated that when the bioflocculant was introduced, the total interaction energy decreased sharply, resulting in higher flocculation efficiency. The model provided a detailed interpretation of conceivable mechanism of microalgae bioflocculation by γ-PGA broth. The pilot-scale study revealed that this innovative harvesting method is scalable and feasible for *in-situ* flocculation. The flocculation efficiency >98% was achieved at both pilot-scale and *in-situ* treatment. Moreover, biochemical composition analysis revealed that there was no significant difference in total lipids recovered from biomass harvested either by centrifugation or bioflocculation, suggesting that this bioflocculation technology is a worthy microalgae harvesting method for algal based biofuel production. The bioflocculation process is easy to operate, cost-efficient, environmentally friendly and as effective as chemical flocculation processes applied industrially. The γ-PGA bioflocculant produced by *B. licheniformis* CGMCC 2876 demonstrated high performance for optimal microalgae recovery and can be applied in commercial-scale microalgae harvesting. In addition, bioflocculation process cost could greatly be reduced by *in-situ* operation as no investment cost is needed for a separate flocculation tank and mixing device. Furthermore, for effective application of dewatering technology for the production of low value products such as biofuels, a two-stage microalgae dewatering system was developed. The performance and economic assessment of different two-stage dewatering systems revealed that bioflocculation coupled with tangential flow filtration (TFF) was a promising dewatering system with total energy input of 0.041 kWh, 0.05 kg CO_2 emissions and a cost of \$ 0.0043 for producing 1 kg of microalgae biomass.

Keywords: Microalgae, Harvesting, Dewatering, Bioflocculation, Bacterial bioflocculant, Pilot-scale.

摘 要

随着世界对食品、饲料、生物燃料和化工产品需求的日益增长，微藻成为了被广泛接受的最具有前景的原材料之一。然而，微藻细胞个体微小，培养密度低，藻体难以高效收集，影响了微藻产品的生产和商业化。本研究从经济和环保的角度出发，建立了一种使用生物絮凝剂絮凝收集藻体的方法。方法使用地衣芽孢杆菌 CGMCC 2876 生产的聚谷氨酸作为生物絮凝剂，絮凝并收集淡水藻体，同时使用 zeta 电位测定和 DLVO 经典理论对絮凝过程进行了分析研究，并对絮凝操作的可放大性和原位操作的可行性进行了实验评估。此外，本研究对所收集微藻的生化组成进行了测定，分析了其作为生物燃料的可行性。实验结果表明，微藻的絮凝收率取决于藻液的初始 pH 值。通过条件优化得出，当初始 pH=3.0，絮凝剂添加量为 2.5 mL/L，在 150 rpm 下搅拌 1 min，随后 80 rpm 搅拌 2 min 的条件下，藻体收率可达 99%。微藻间的相互作用能和 zeta 电位相关，使用 DLVO 理论模型分析的结果表明，当加入生物絮凝剂后，藻液总相互作用能迅速下降，从而提升了絮凝率，很好的说明了聚谷氨酸絮凝微藻的机制。本研究所用方法在中式规模和原位操作的情况下均能获得 98%以上的藻体收率，说明这种微藻回收方法具备可放大性和原位操作性能。另外，生化组分分析结果表明，通过絮凝所得的藻体与离心所得藻体中总油脂的含量没有明显差别，这说明生物絮凝剂收集微藻的技术适用于微藻生物燃料的生产，且易于操作，经济高效，对环境友好，与化学絮凝剂效果相当。地衣芽孢杆菌 CGMCC 2876 生产的生物絮凝剂聚谷氨酸拥有优良的藻体絮凝特性，易于规模化生产，且原位絮凝操作无需单独的絮凝池和混合装置，能够大大节省藻体收集成本，具有良好的商业应用价值。另外，为了有效地生产低附加值产品，如生物燃料，本研究建立了一种生物絮凝和错流过滤耦合的两段式微藻脱水系统，与其他两段式脱水系统相比，此方法每生产 1 kg 微藻生物质，仅耗费 0.041 kWh 能量和 0.0043 美元，同时可吸收 0.05 kg CO_2。

关键词：微藻；回收；脱水；生物絮凝；细菌生物絮凝剂；中试实验。

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ASACF: Air sparging assisted coagulation flocculation

BSA: Bovine Serum Albumin

CF: Concentration factor

COD: Chemical oxygen demand

CTAB: Cetyl trimethylammonium bromide

DCW: Dry cell weight

DLVO: Derjaguin-Landau-Verwey-Overbeek

ECF: Electro-coagulation flocculation

EPS: Extracellular polysaccharides

FE: Flocculation efficiency

FR: Flocculating rate

GC: Gas chromatography

GHG: Greenhouse gas

HPAEC: High-performance Anion exchange chromatography

HPLC: High-performance liquid chromatography

ISO: International organization of r standardization

LCA: Life cycle assessment

N.D: Not determined

PBR: Photobioreactors

SCFE: Supercritical fluid extraction

ST: Settling time

TFF: Tangential flow filtration

TSS: Total suspended solids

Γ-PGA: Poly γ-glutamic acid

LIST OF FIGURES

LIST OF TABLES